Network Formation and Crosslinking of Cytoskeletal Polymers and Filamentous Viruses

1. Polyelectrolyte properties of filamentous biopolymers
2. Inactivation of antimicrobial factors by F-actin, DNA, and Pf1 virus
3. Gel formation by Pf1 mediated by low concentrations of multivalent counterions - fragile crosslinks and rapid reformation

Robert Bucki
Eric Wong
Katrina Cruz
Penn

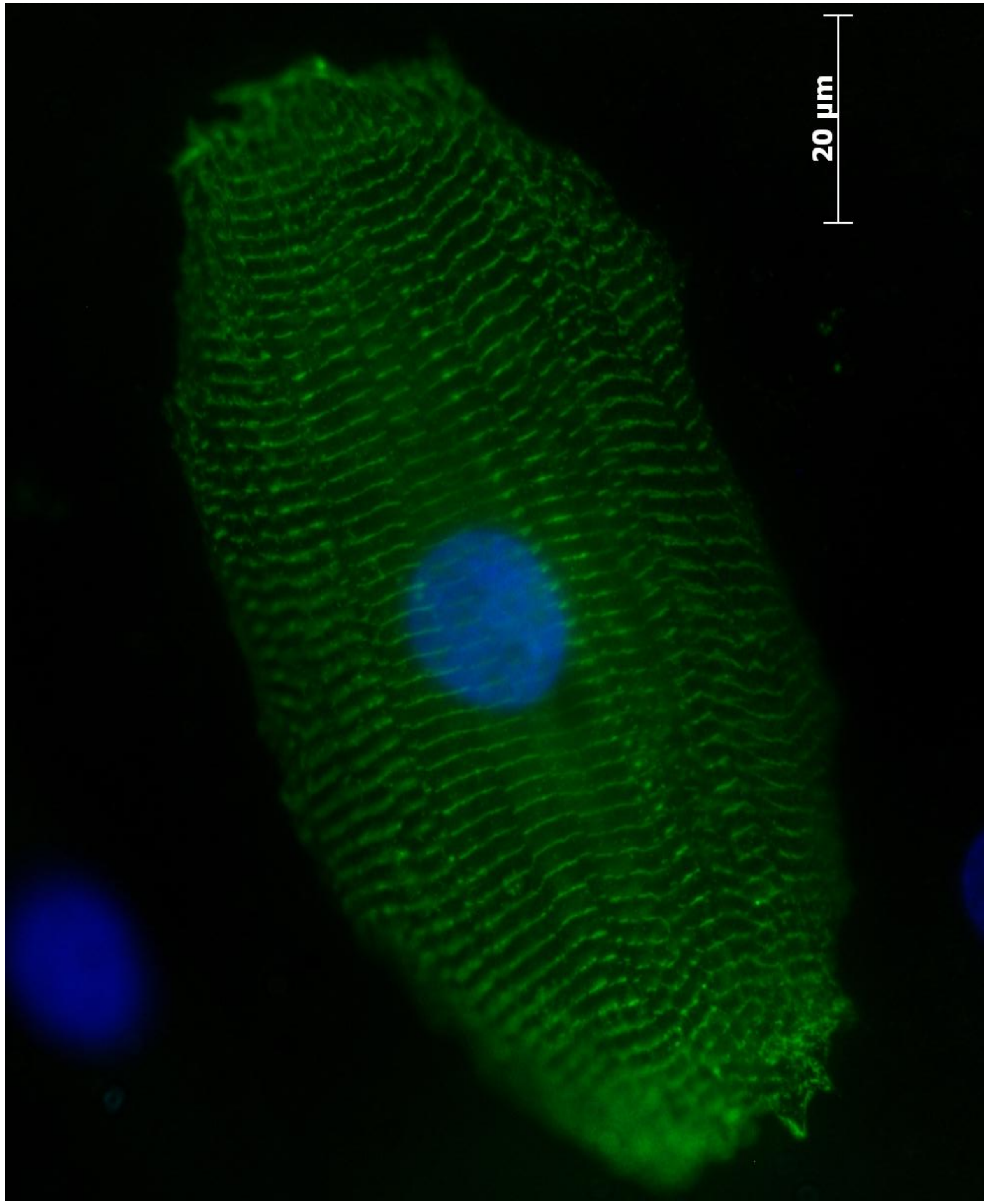
Katarzyna Leszczyńska
Bialystok

Liesbeth Huisman
Leiden/Penn

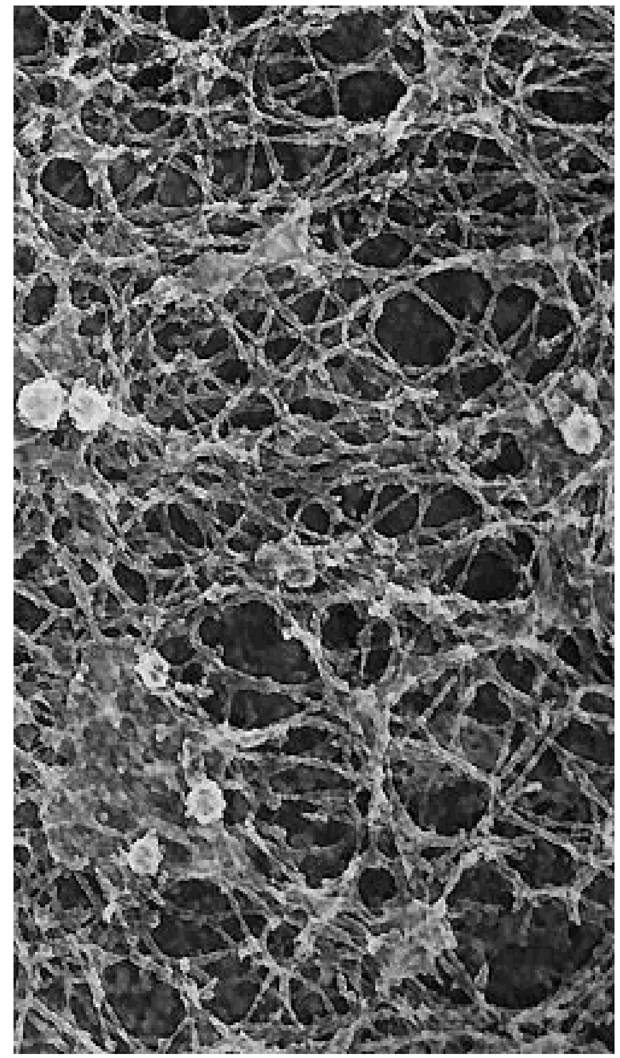
Andrejs Cebers
Andris Zeltinsh
Riga

Janmey/KITP 110526

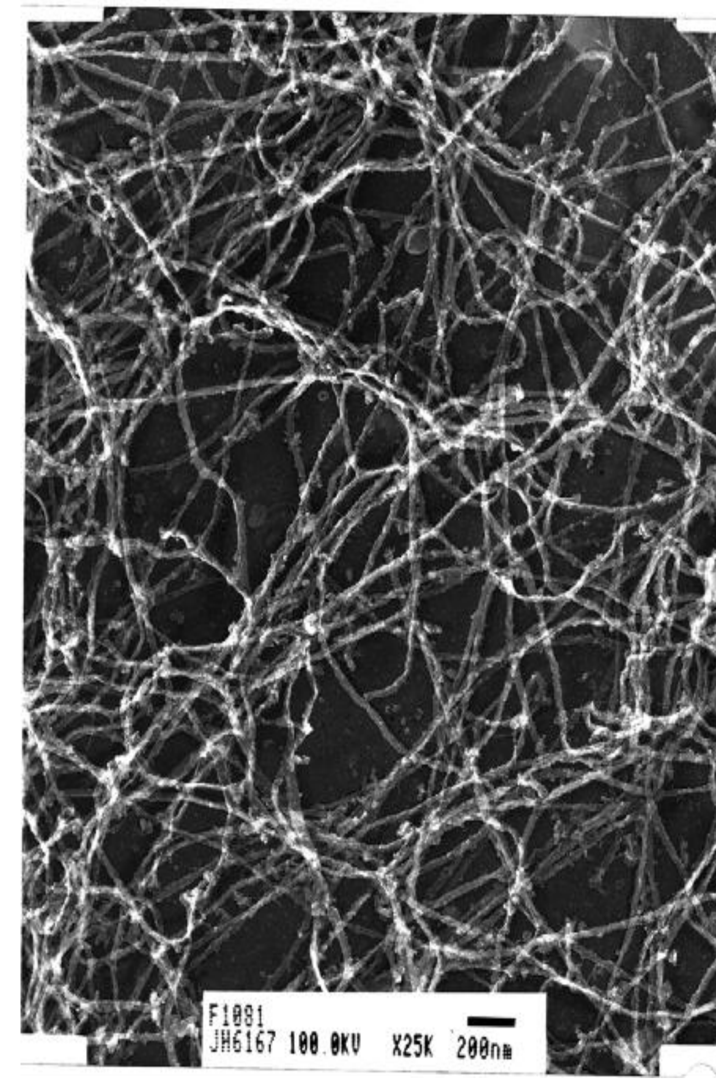
Cells are filled with long filamentous anionic filaments that can inactivate antimicrobial factors



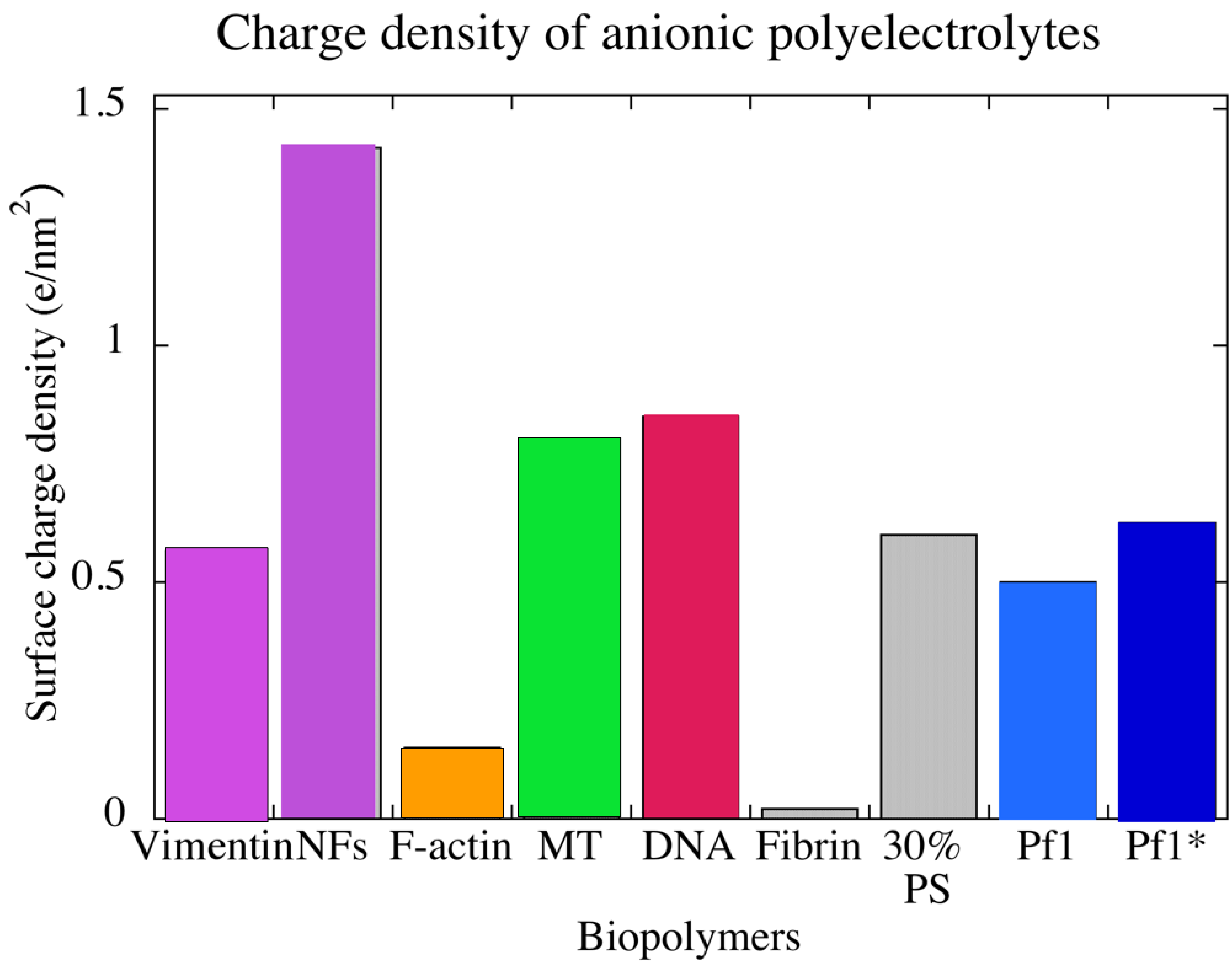
Cardiac myocyte



Cortical actin gel in macrophage



neurofilaments


Charge density of anionic polyelectrolytes
Surface charge density (e/nm^2)
1.5
1
0.5
0
Vimentin
NFs
F-actin
MT
DNA
Fibrin
30% PS
Pf1
Pf1*
Biopolymers

Human immune system

Innate

Acquired

Cellular: monocytes
neutrophils
NK cells

B lymphocytes
T lymphocytes

al: complement
lysozyme
antimicrobial peptides

immunoglobulin

Relatively non- specific
but ubiquitous

Very highly specific

Antimicrobial peptides

Made by nearly all species

Present on skin, eye, oral cavity, respiratory, urogenital and digestive tracts

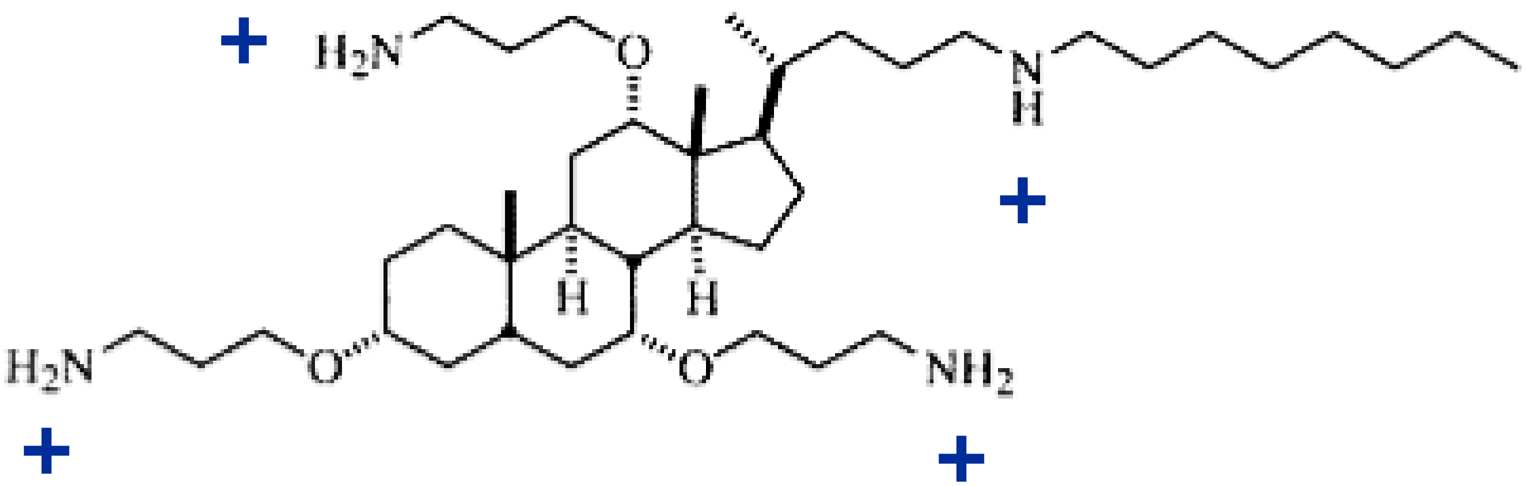
Positive charge, amphipathic character

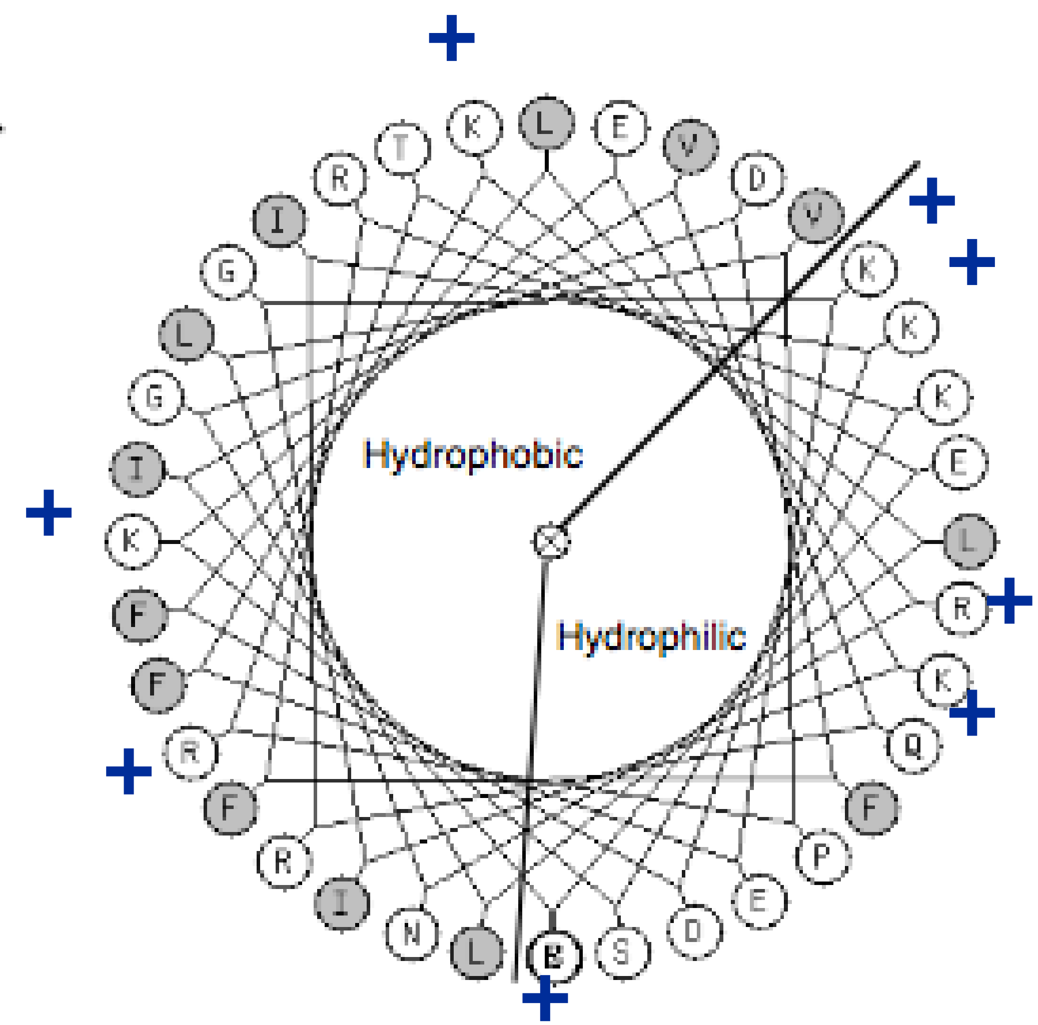
Targeted to bacterial membranes by electrostatic interactions

Bacterial mutation inefficient to develop resistance to AMPs

Antimicrobial peptides and steroids

Ceragenin CSA-13:

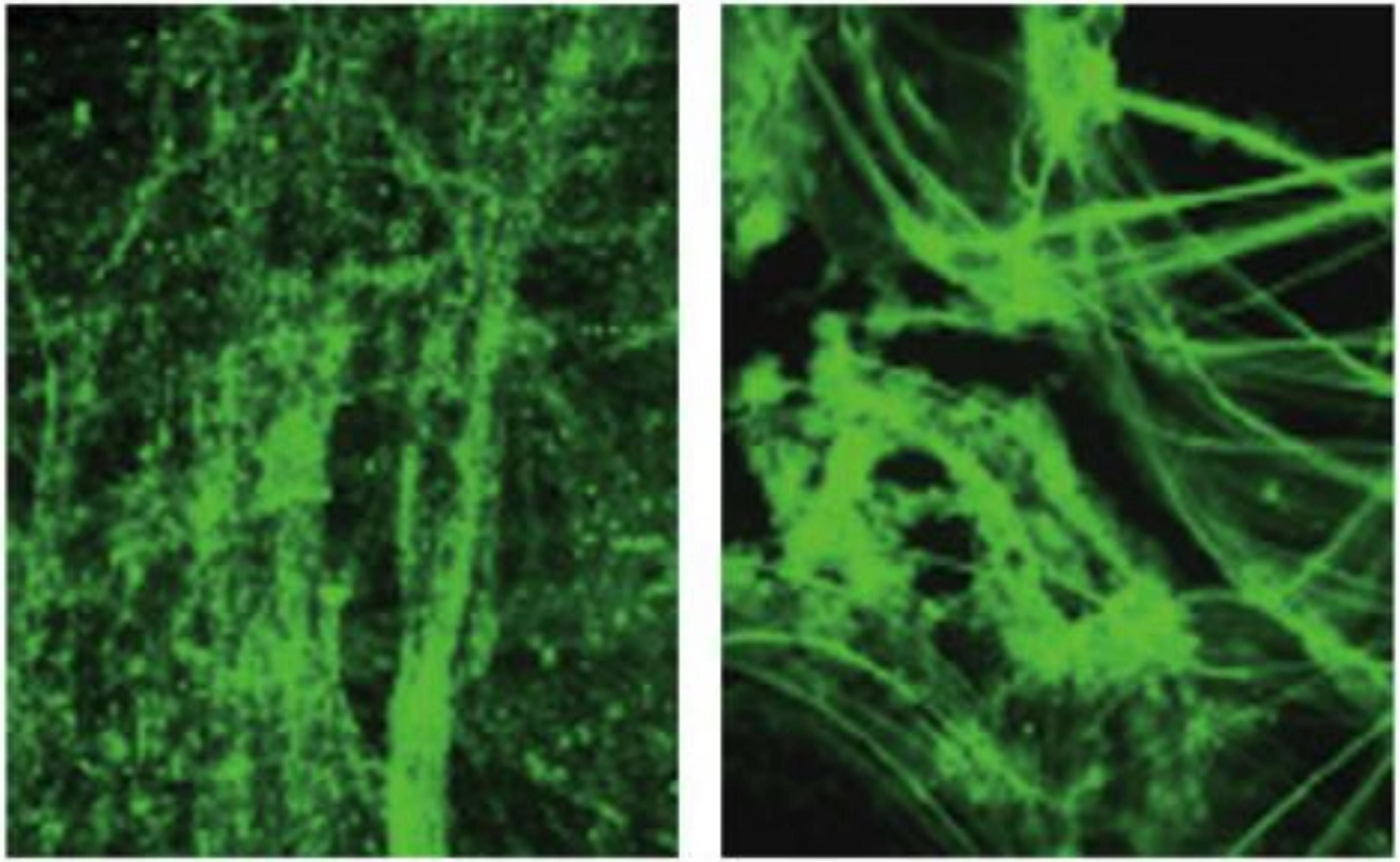




Cathelicidin LL-37:

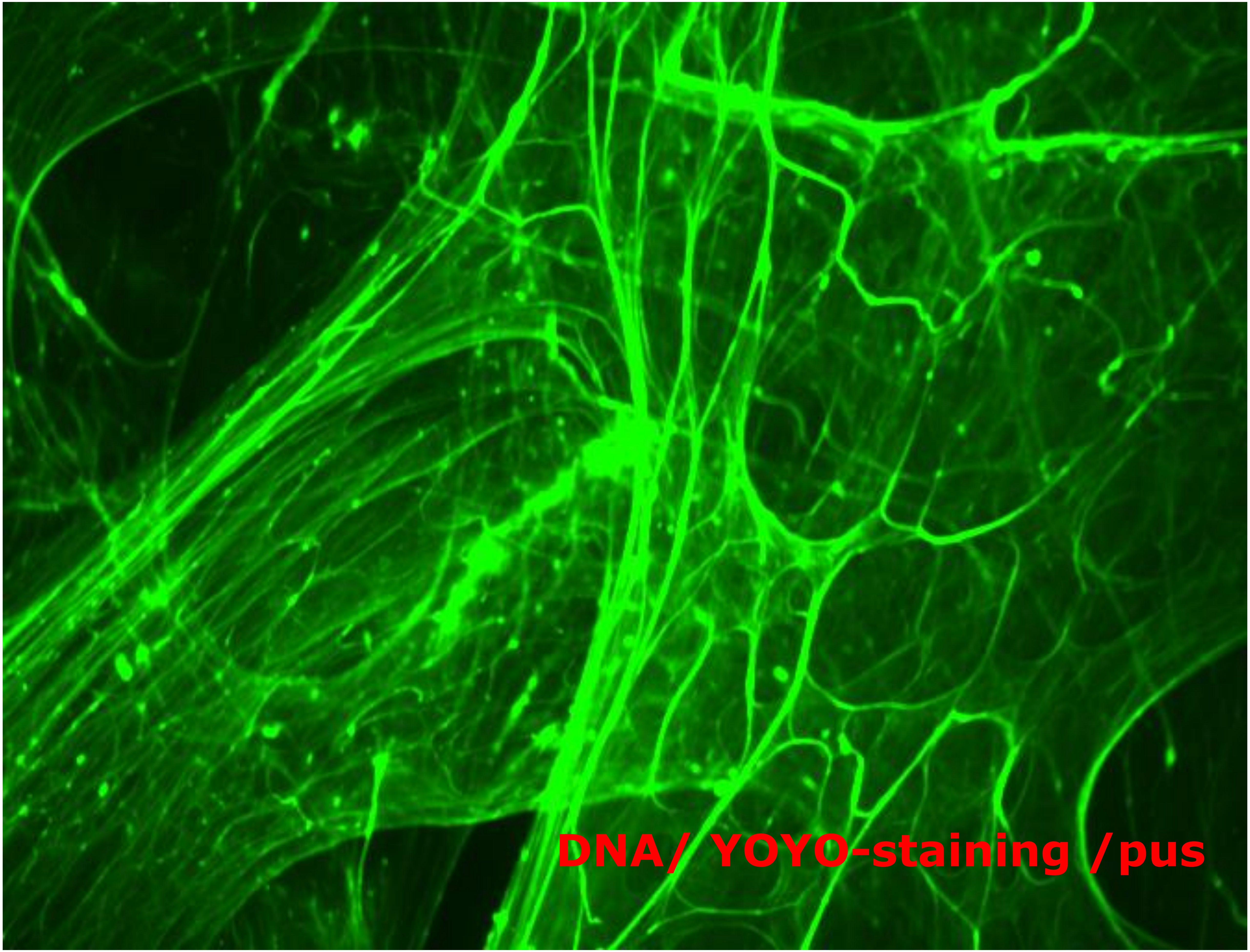
LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES

When cells rupture, anionic polymers are released into extracellular fluids like CF sputum



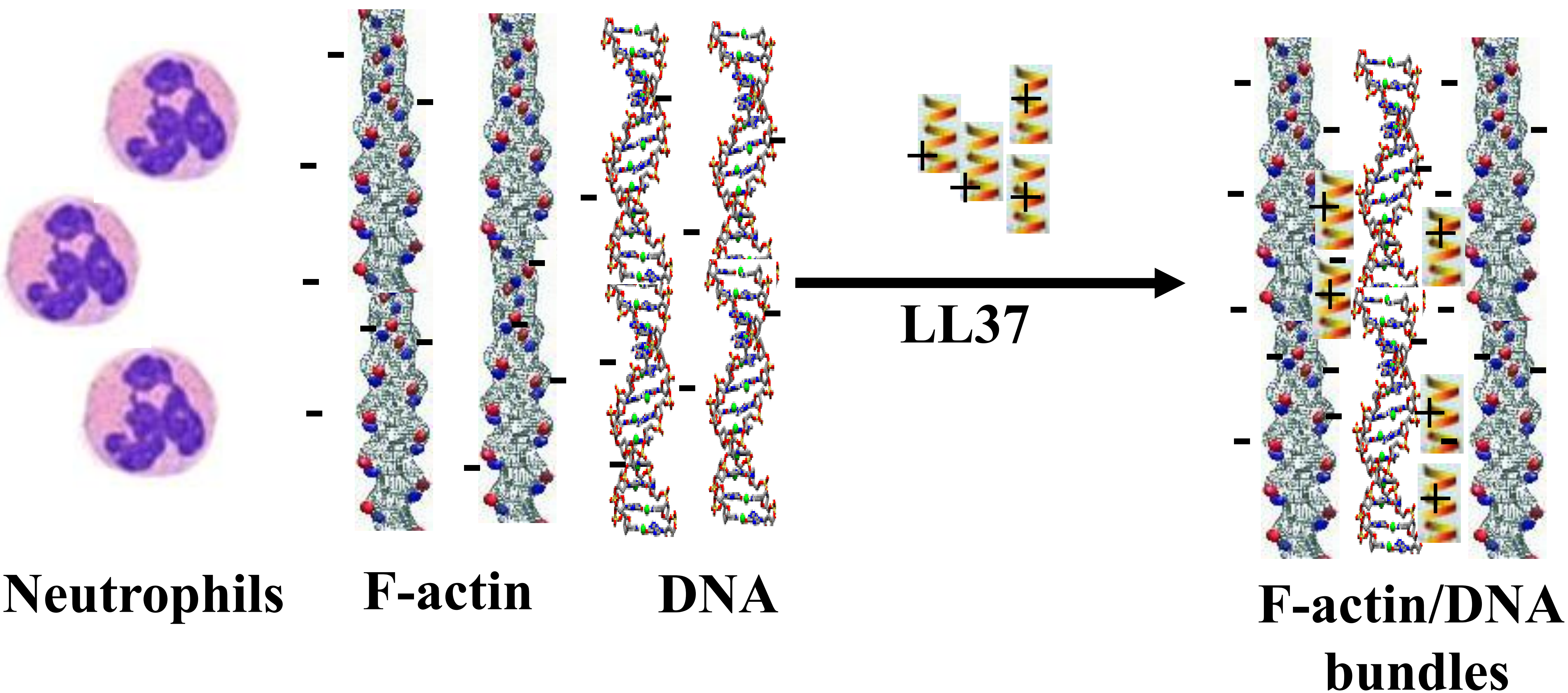
F-actin DNA

Weiner, D. J., R. Bucki, PAJ. 2003. The antimicrobial activity of the cathelicidin LL37 is inhibited by F-actin bundles and restored by gelsolin. Am J Respir Cell Mol Biol 28:738-745.


DNA/ YOYO-staining /pus

Anionic polyelectrolytes inactivate antimicrobial factors

Inhibition of LL37 antibacterial activity by F-actin/DNA bundles



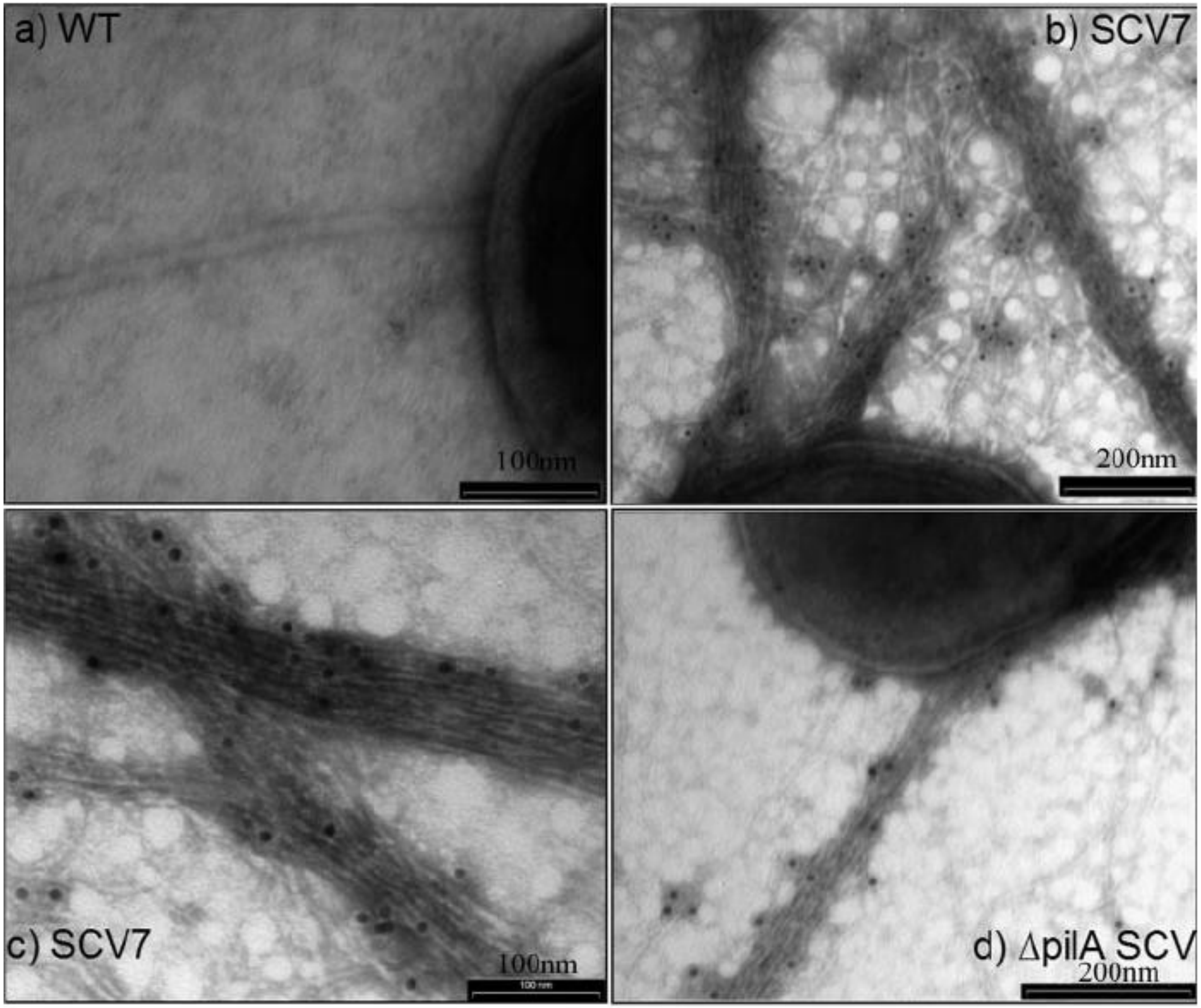
Host-derived polyelectrolytes are not the only factors that inhibit antimicrobial agents. Bacteria produce their own anionic filaments.

Table 1 Genes differentially expressed in *P. aeruginosa* biofilms

P. aeruginosa ORF	Number	Fold activation (mean ± s.e.m.)
Bacteriophage genes		
Coat protein B of bacteriophage Pf1	PA0723	83.5 ± 10.3
Hypothetical protein of bacteriophage Pf1	PA0722	64.2 ± 5.6
Helix-destabilizing protein of bacteriophage Pf1	PA0720	35.2 ± 2.7
Hypothetical protein of bacteriophage Pf1	PA0721	26.6 ± 4.1
Protein of bacteriophage Pf1	PA0718	22.6 ± 2.9
Hypothetical protein from bacteriophage Pf1	PA0727	14.6 ± 2.4
Probable coat protein A of bacteriophage Pf1	PA0724	10.1 ± 0.6
Hypothetical protein of bacteriophage Pf1	PA0725	9.9 ± 1.1
Hypothetical protein of bacteriophage Pf1	PA0726	8.9 ± 0.5

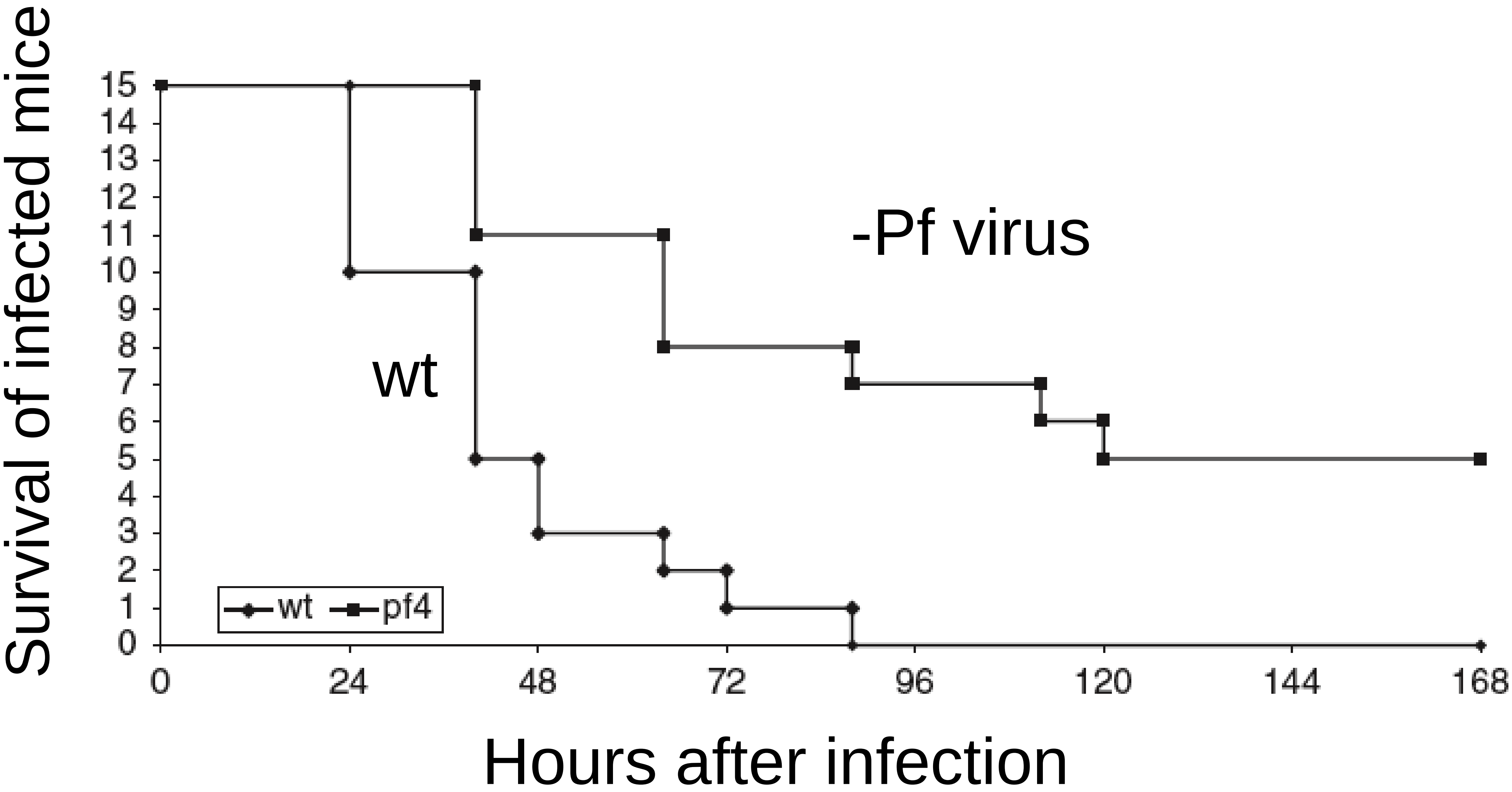
Whiteley M, Bangera MG, Bumgarner RE, Parsek MR, Teitzel GM, et al. (2001) Gene expression in Pseudomonas aeruginosa biofilms. Nature 413: 860-864.

Pf1 and related bacteriophages grow from surface of *P. aeruginosa*



Webb JS, Lau M, Kjelleberg S (2004) Bacteriophage and phenotypic variation in Pseudomonas aeruginosa biofilm development. J Bacteriol 186: 8066-8073.

Pf virus enhances the virulence of Pseudomomas aeruginosa



Rice SA, Tan CH, Mikkelsen PJ, Kung V, Woo J, et al. (2009) The biofilm life cycle and virulence of Pseudomonas aeruginosa are dependent on a filamentous prophage. ISME J 3: 271-282.

TAXONOMY

Group II: ssDNA viruses

Family:

Inoviridae

Genus:

Inovirus

Plectrovirus

ETYMOLOGY

Ino: from Greek, 'muscle'

SPECIES

Type:

Enterobacteria phage M13 (M13)

Acholeplasma phage L51 (L51)

Main:

Pseudomonas phage Pf1 (Pf1)

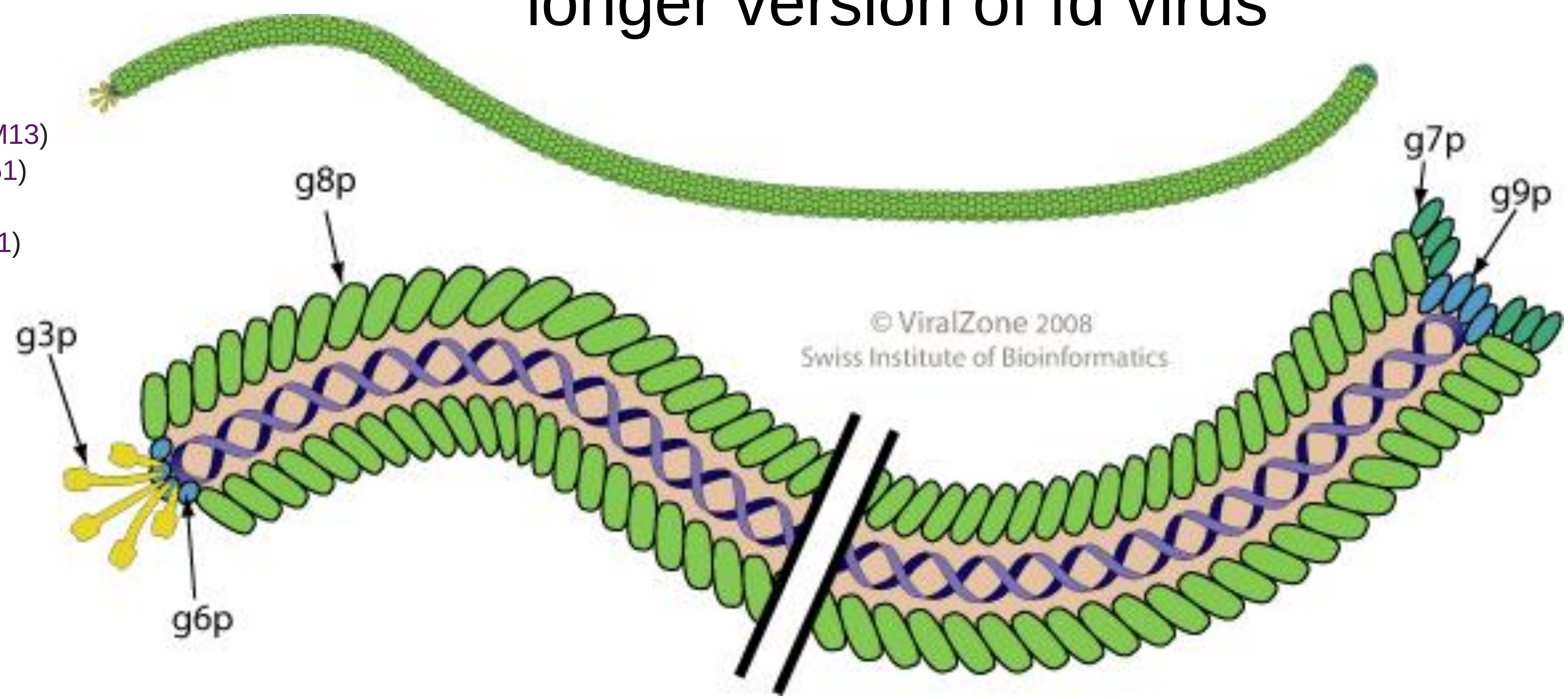
Pf1 virus

L = 2 µm, d = 6 nm

Lp = 2 µm

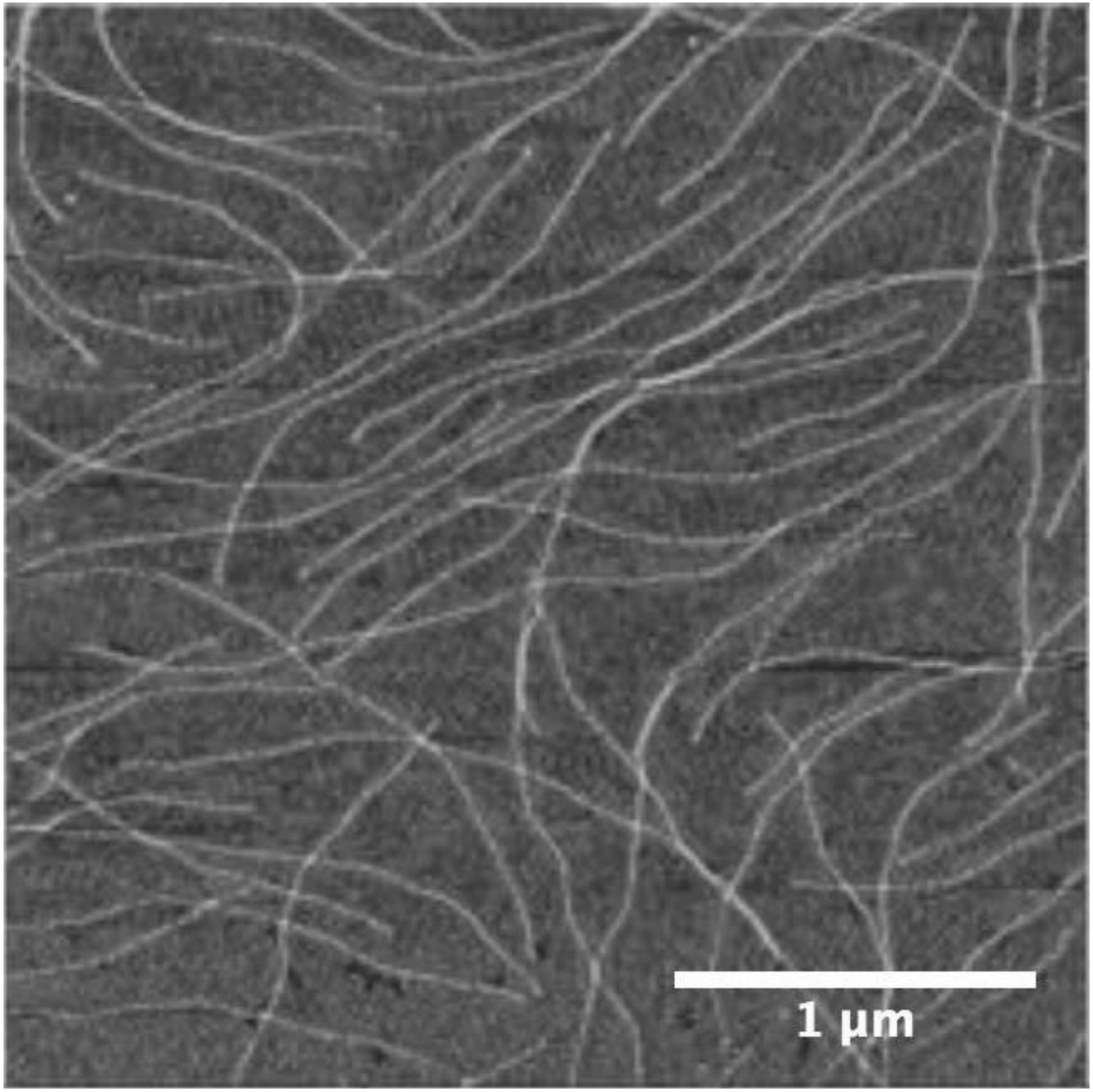
0.5 neg. charges/nm^2

longer version of fd virus

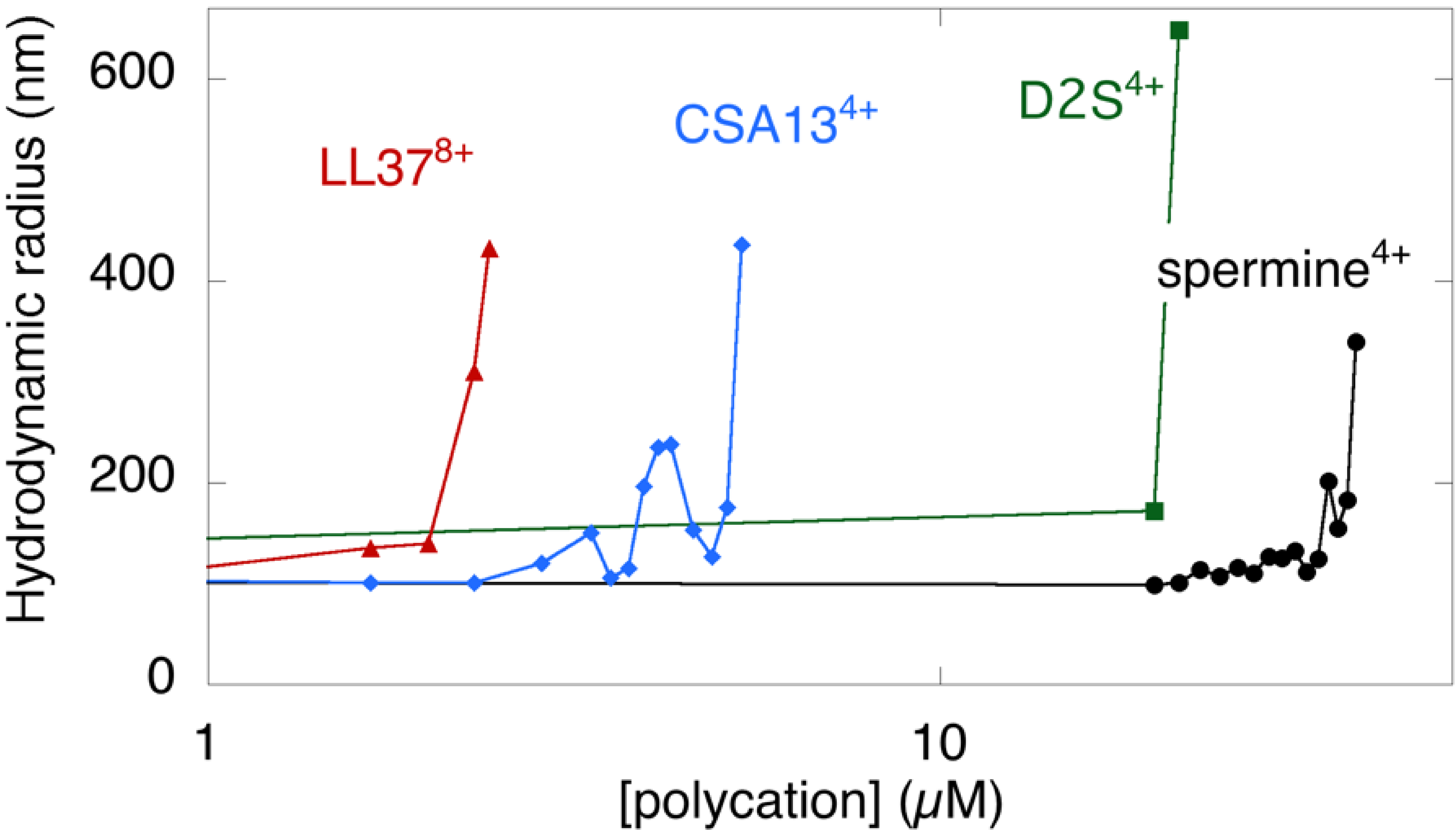


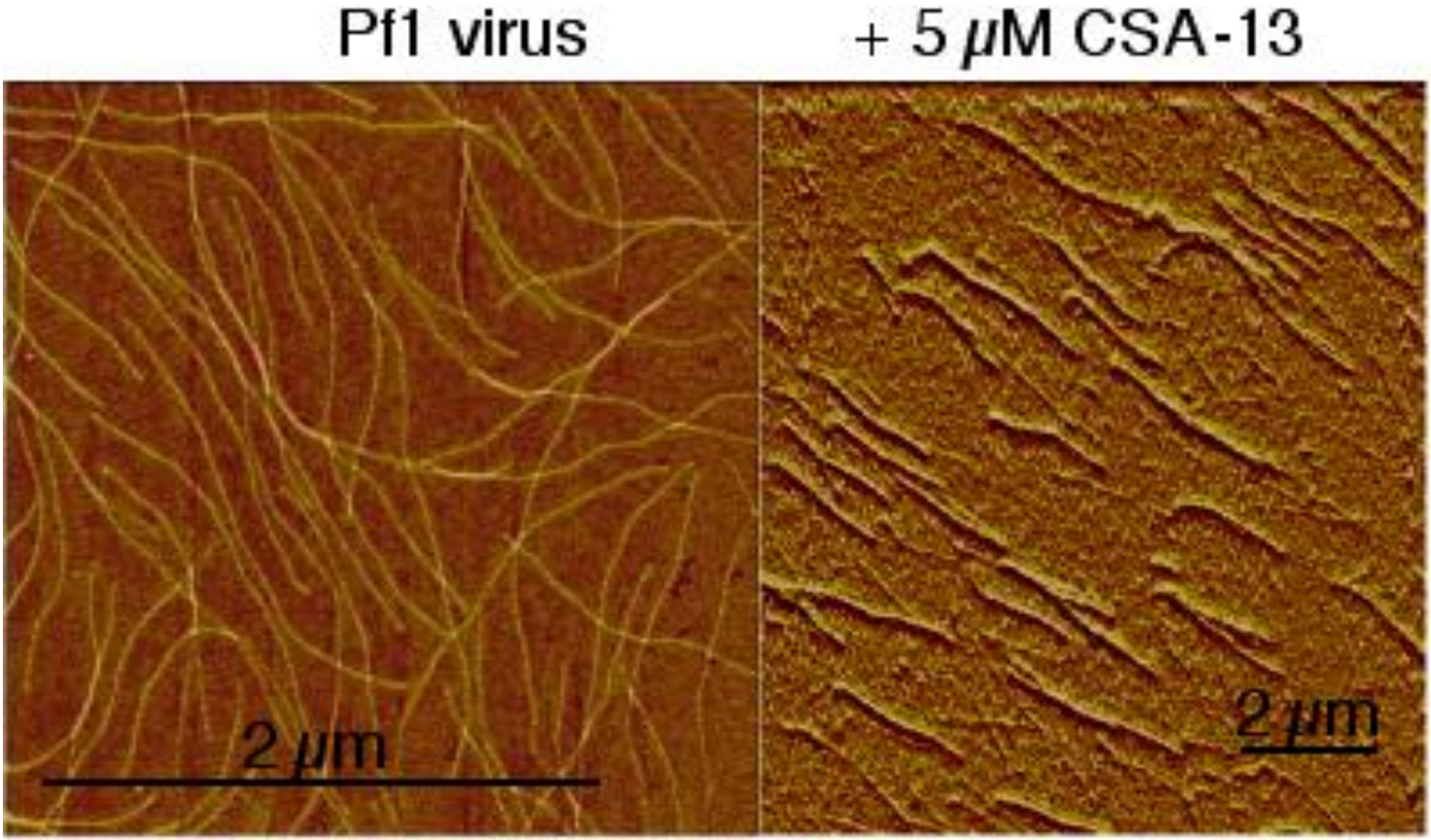
expasy.org/viralzone/all_by_protein/113.html

AFM image of Pf1 virus

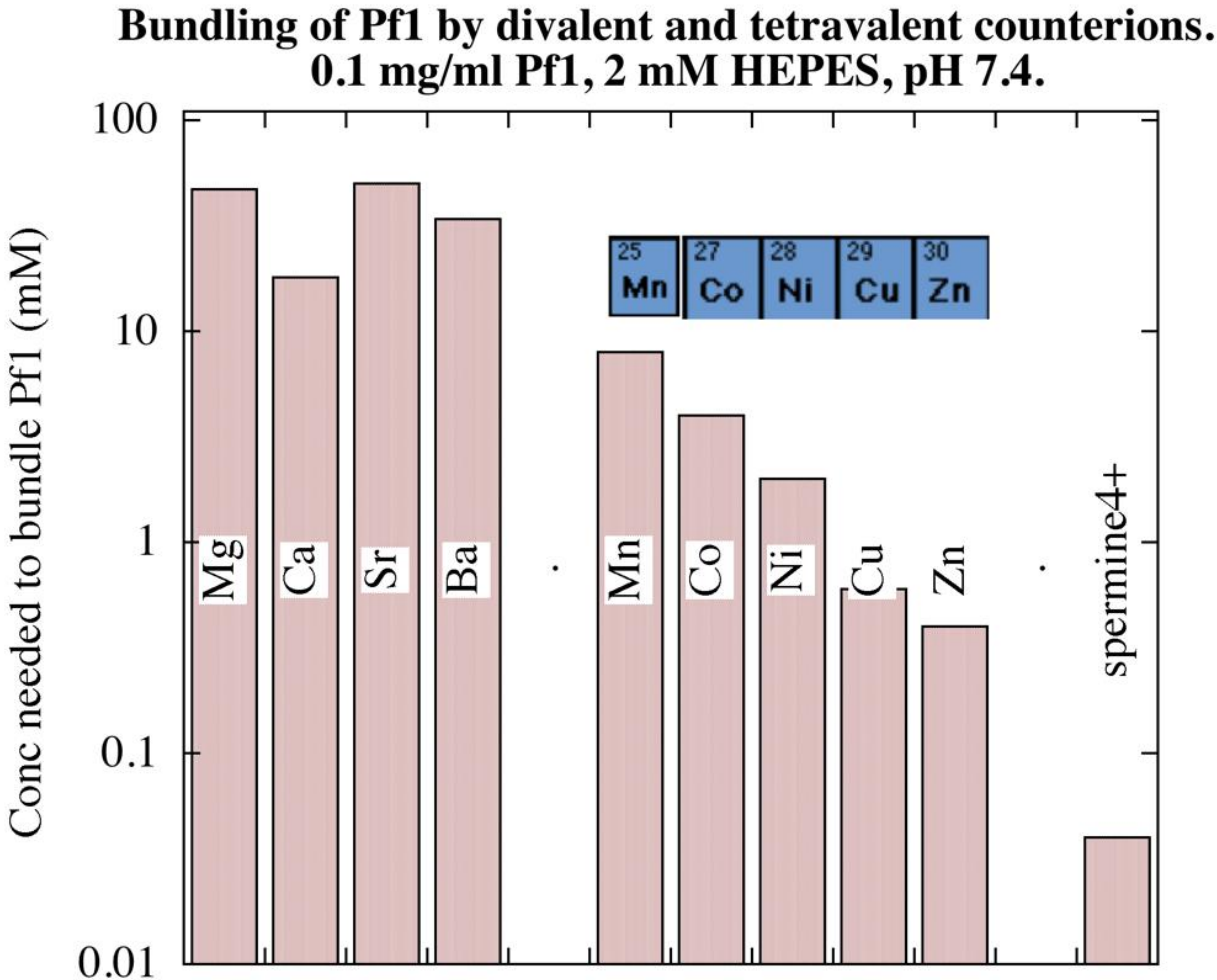


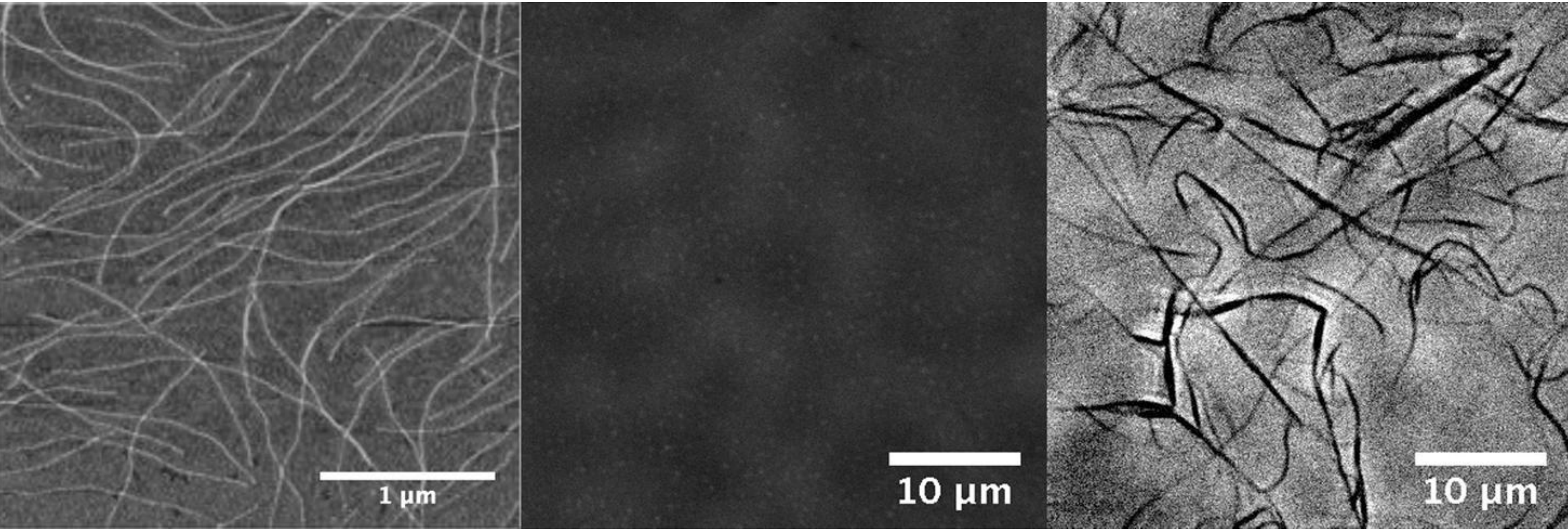
Cationic antimicrobial factors interact strongly with Pf1 virus



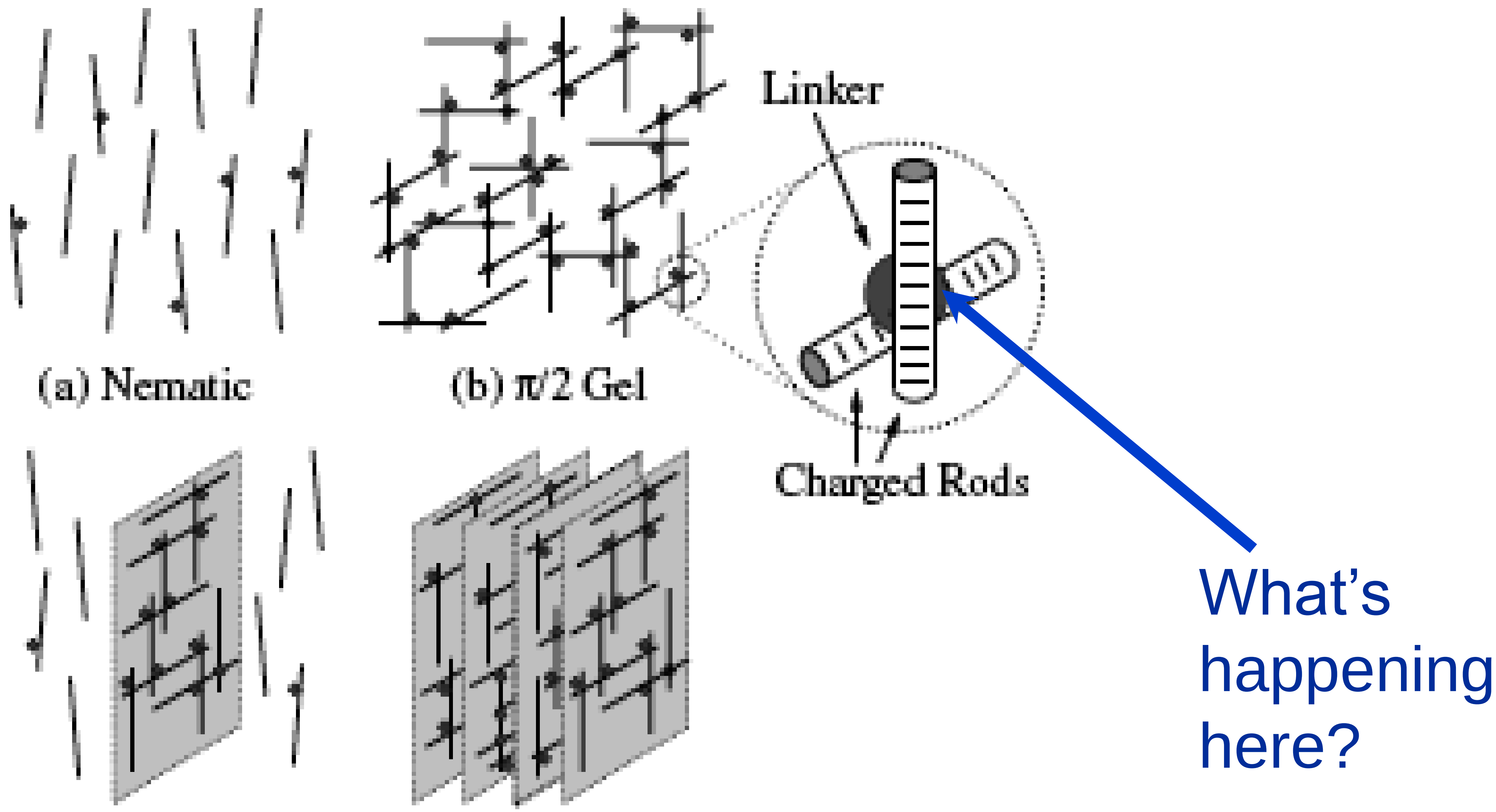


Antimicrobials like CSA-13 don’t work when in these bundles


Bundling of Pf1 by divalent and tetravalent counterions.
0.1 mg/ml Pf1, 2 mM HEPES, pH 7.4.
Conc needed to bundle Pf1 (mM)
100
10
1
0.1
0.01
Mg
Ca
Sr
Ba
Mn
Co
Ni
Cu
Zn
spermine4+
25
Mn
27
Co
28
Ni
29
Cu
30
Zn


1 μm
10 μm
10 μm

What happens at multivalent counterion concentrations less than what is required for bundling?



(a) Nematic (b) $\pi/2$ Gel (c) A single raft (d) A stack of rafts

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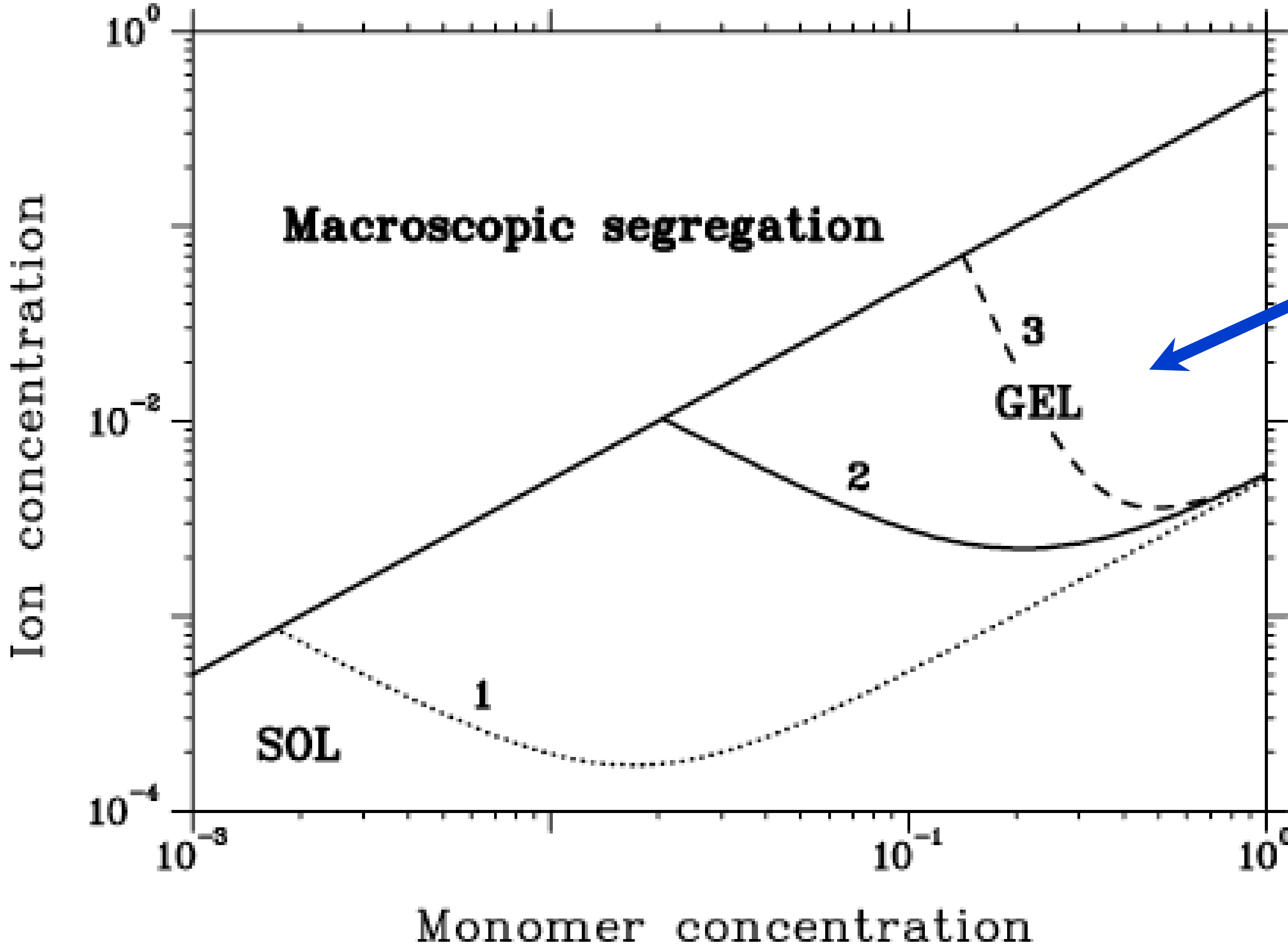
Raft Instability of Biopolymer Gels

Itamar Borukhov[1,2] and Robijn F. Bruinsma[2,3]



Cannot get here with fd virus: too short (800 nm)

FIG. 1. Phase diagram for the solution of polyelectrolyte chains in the presence of divalent ions. Concentrations are given in a^3 units, $l_B/b = 2$, $b = a$, $N = 100$.

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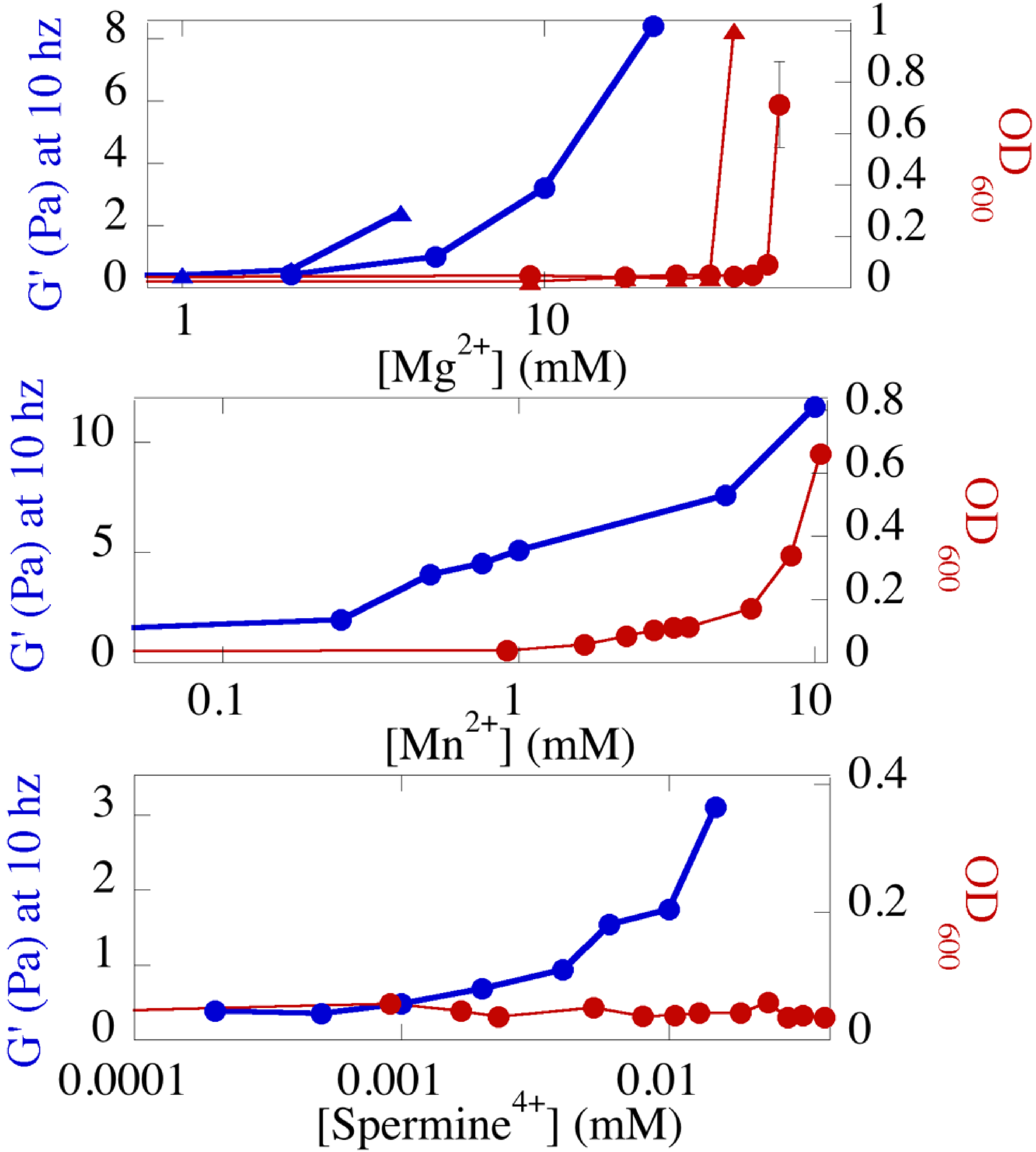
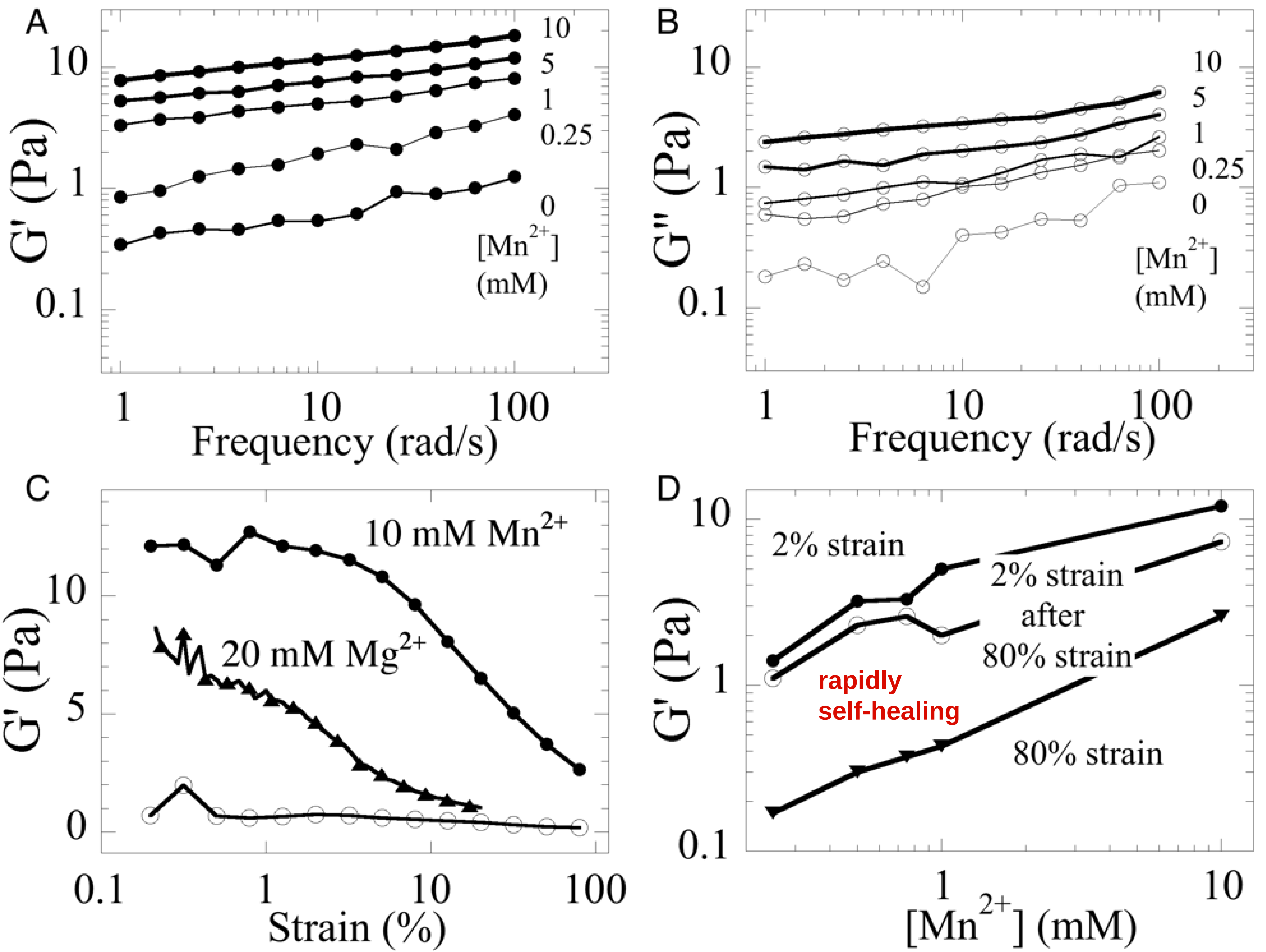
Polyelectrolytes in the Presence of Multivalent Ions: Gelation Versus Segregation

A.V. Ermoshkin and M. Olvera de la Cruz

Gelation of Pf1 occurs at lower counterion concentration than **bundling**



Huisman et al.
Soft Matter 2011


A
G' (Pa)
Frequency (rad/s)
10
5
1
0.25
0
[Mn2+] (mM)
B
G'' (Pa)
Frequency (rad/s)
10
5
1
0.25
0
[Mn2+] (mM)
C
G' (Pa)
Strain (%)
10 mM Mn2+
20 mM Mg2+
D
G' (Pa)
[Mn2+] (mM)
2% strain
2% strain after 80% strain
rapidly self-healing
80% strain

Elastic modulus of 0.04% w/v Pf1 gels measured by fluctuations of 1.3 µm beads during magnetophoresis

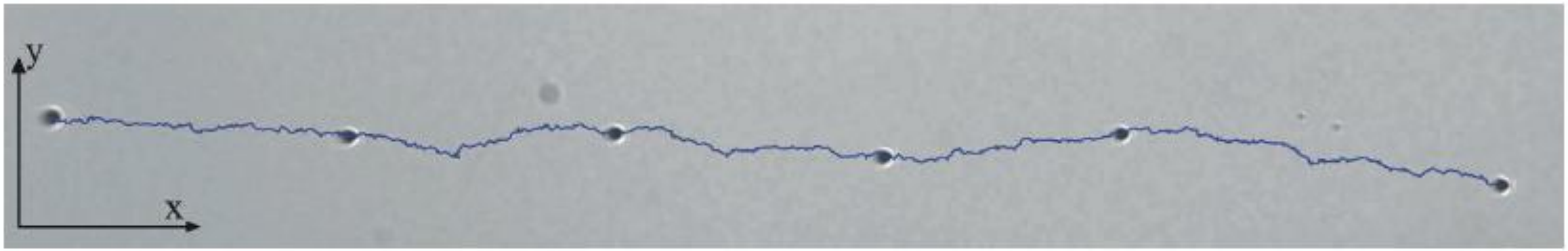
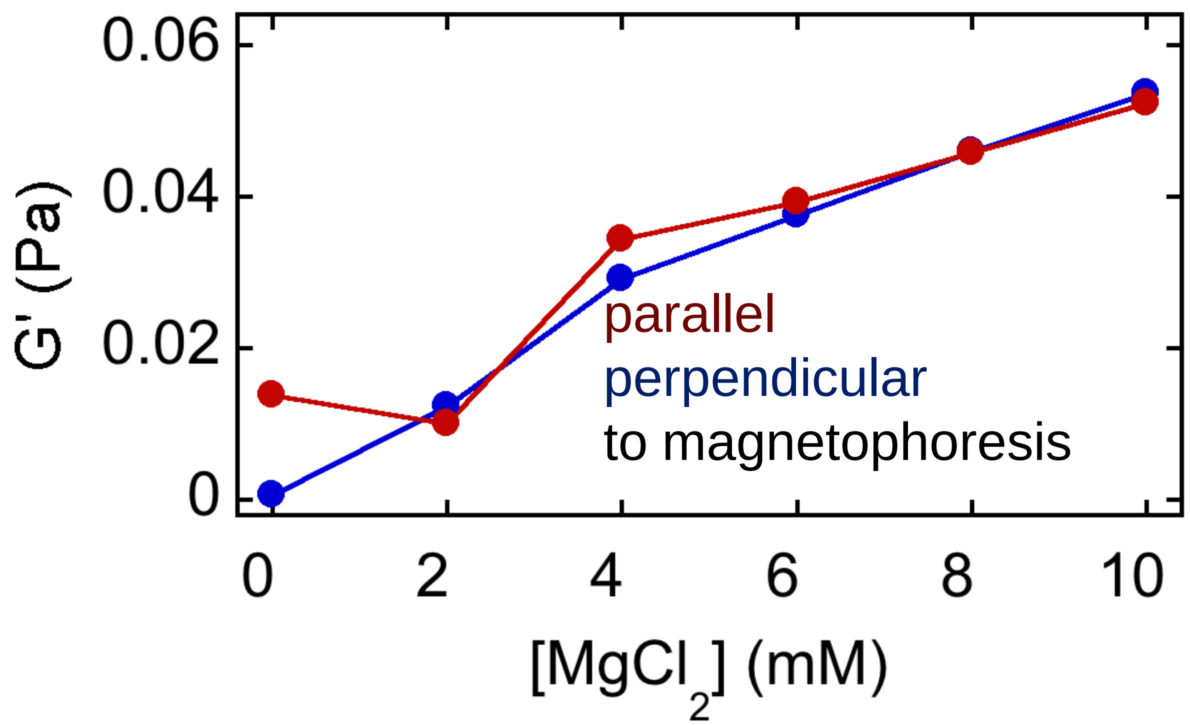
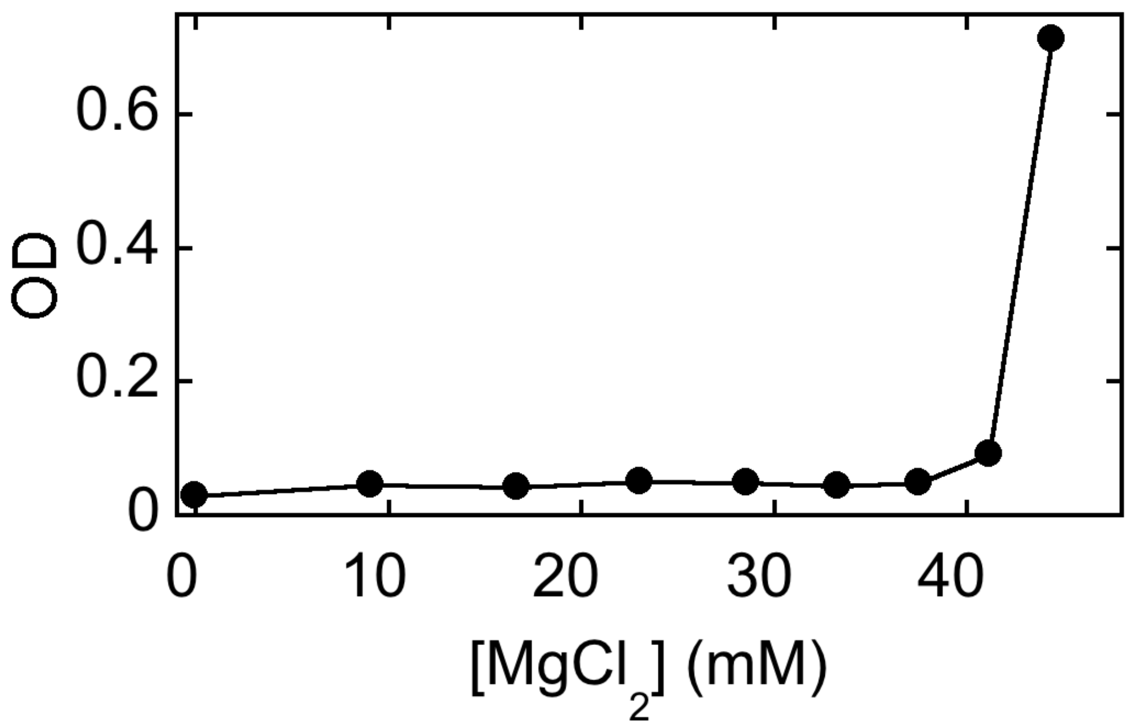


FIGURE 2. Trajectory of a single *Spherotech* 1.31 µm particle.

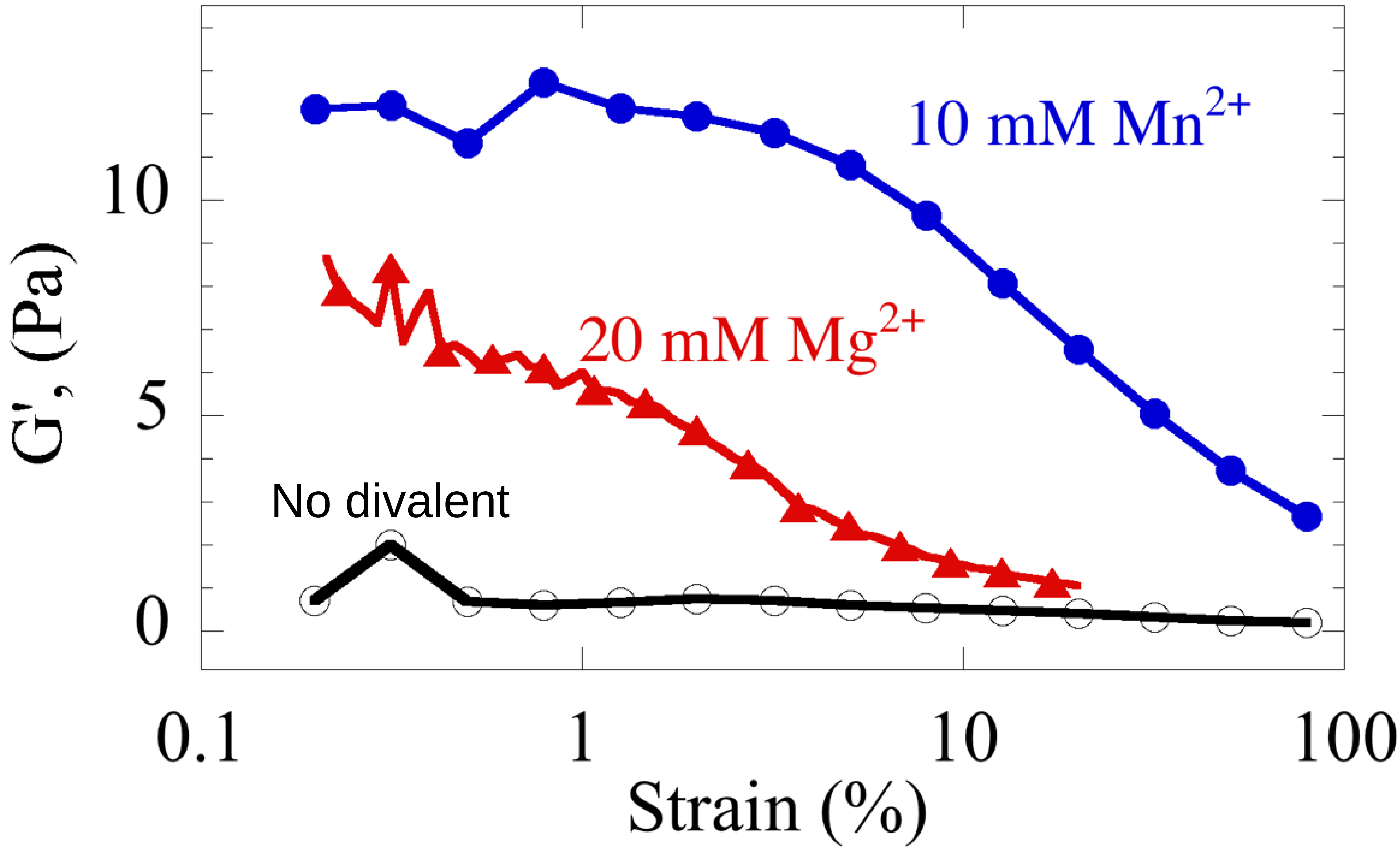
G. Kitenbergs, K. Dzilna, K. Erglis and A. Cebers, *AIP Conference Proceedings*, 2010, **1311**, 141–145.



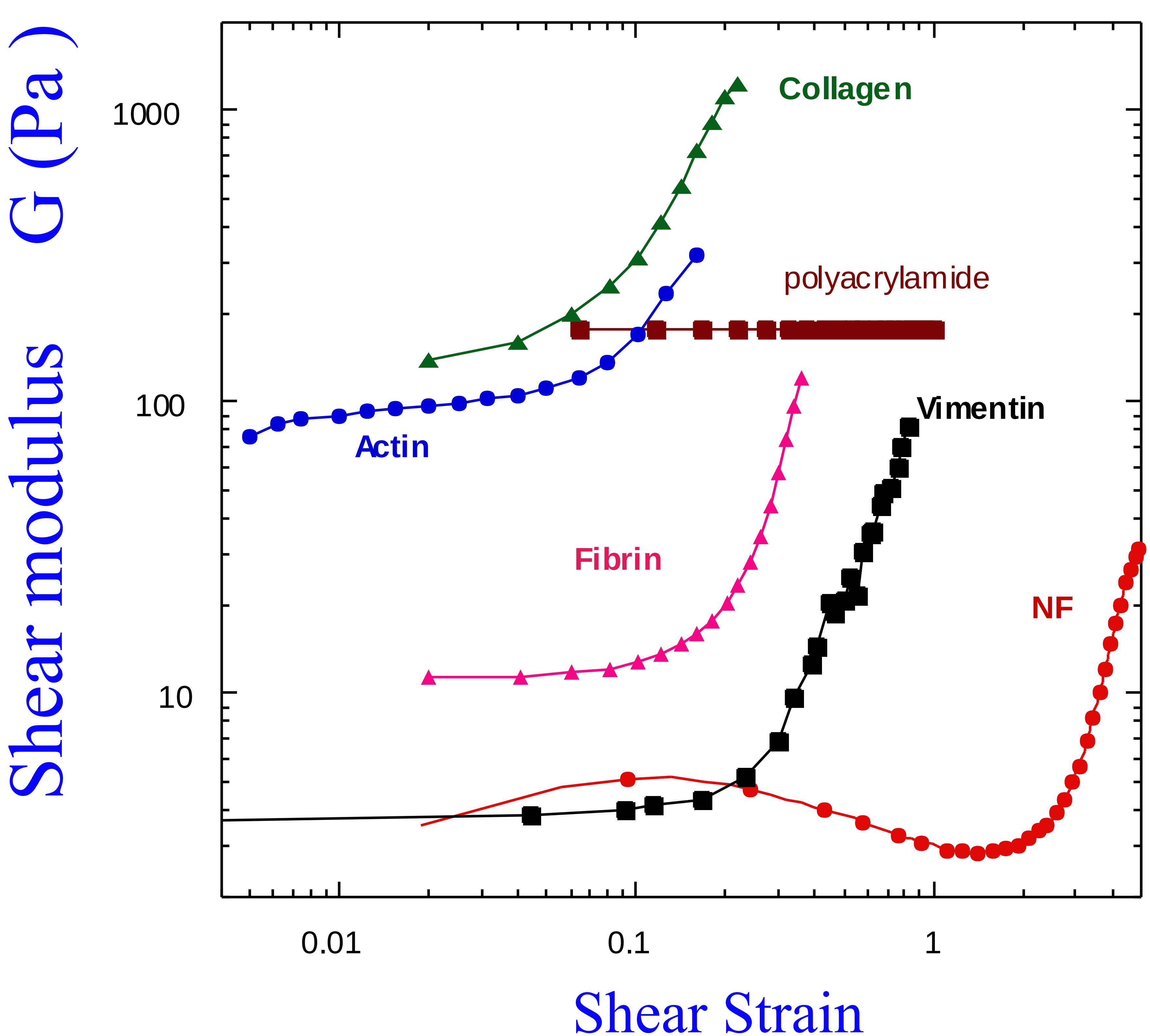


Huisman et al. Soft Matter 2011

Counterion-mediated Pf1 gels are fragile at increasing strain
X-L strength depends on type of counterion



Strongly crosslinked cytoskeletal and extracellualr matrix networks have non-linear (strain-stiffening) elasticity



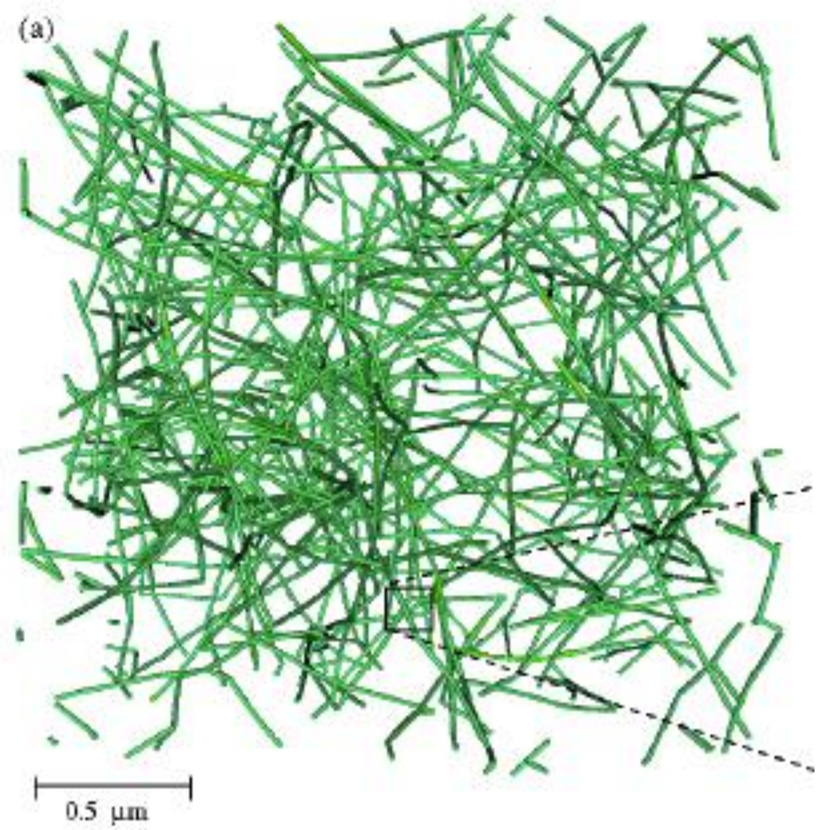
biopolymers
0.1 to 0.3 %

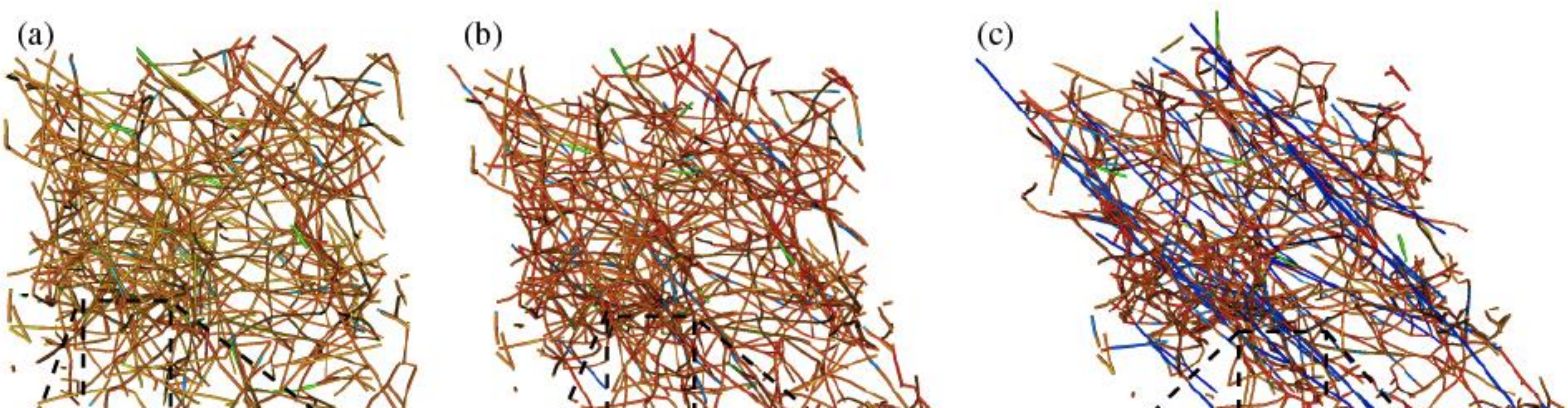
Polyacrylamide: 5%

Storm et al.
Nature, 2005

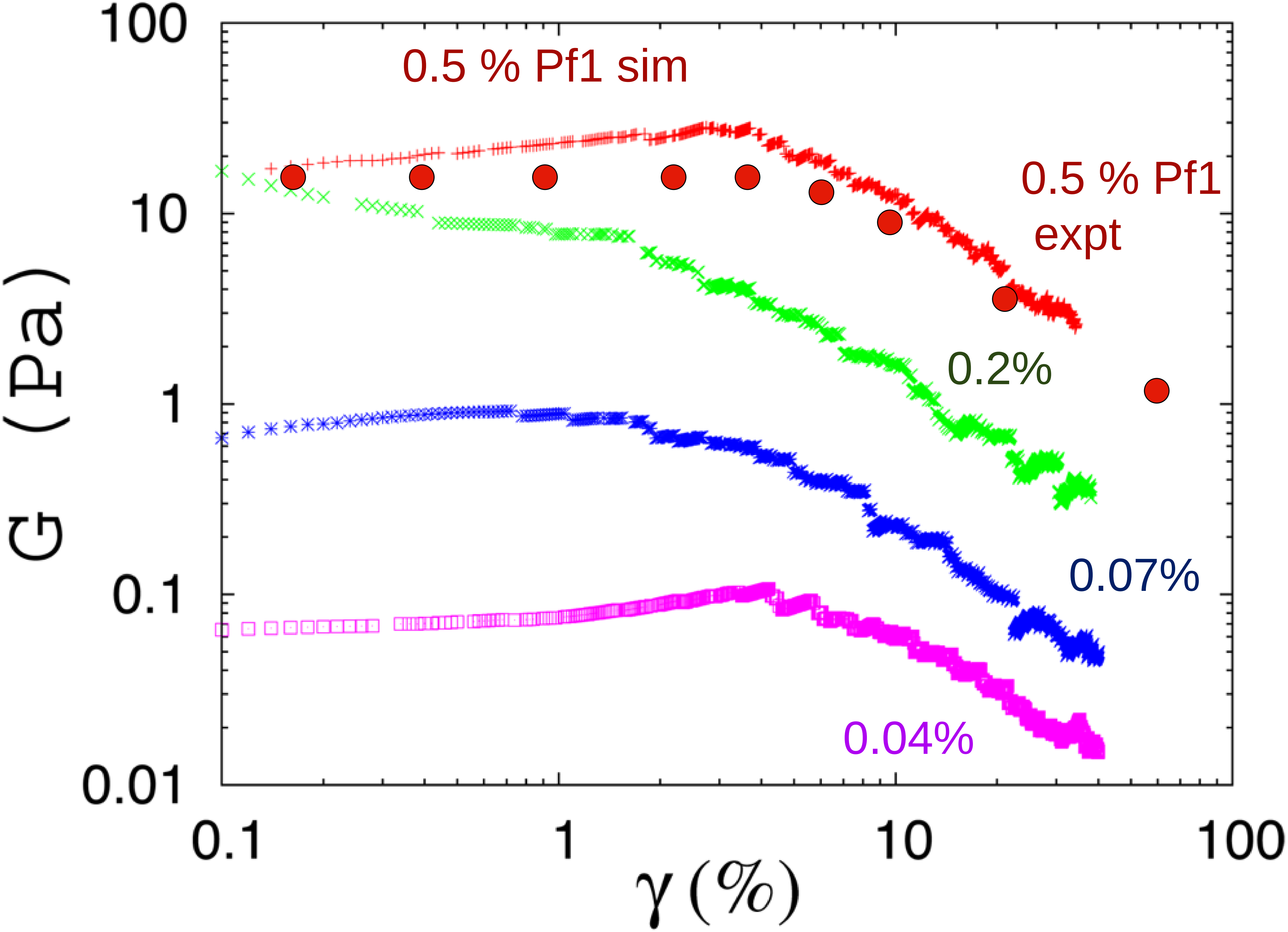
Three-Dimensional Cross-Linked F-Actin Networks: Relation between Network Architecture and Mechanical Behavior

E. M. Huisman, T. van Dillen, P. R. Onck, and E. Van der Giessen





Network simulations with breaking force = 0.05 pN



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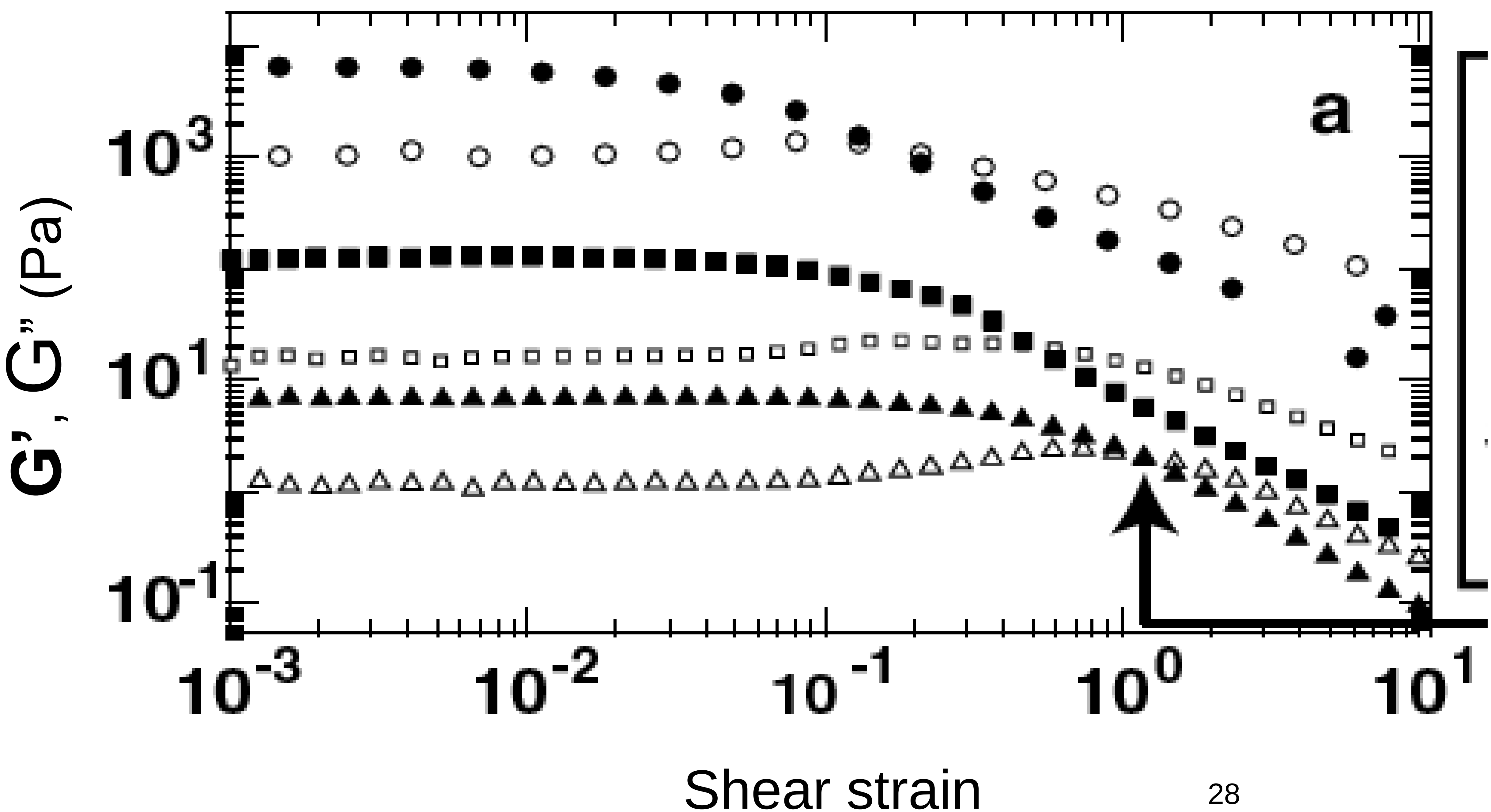
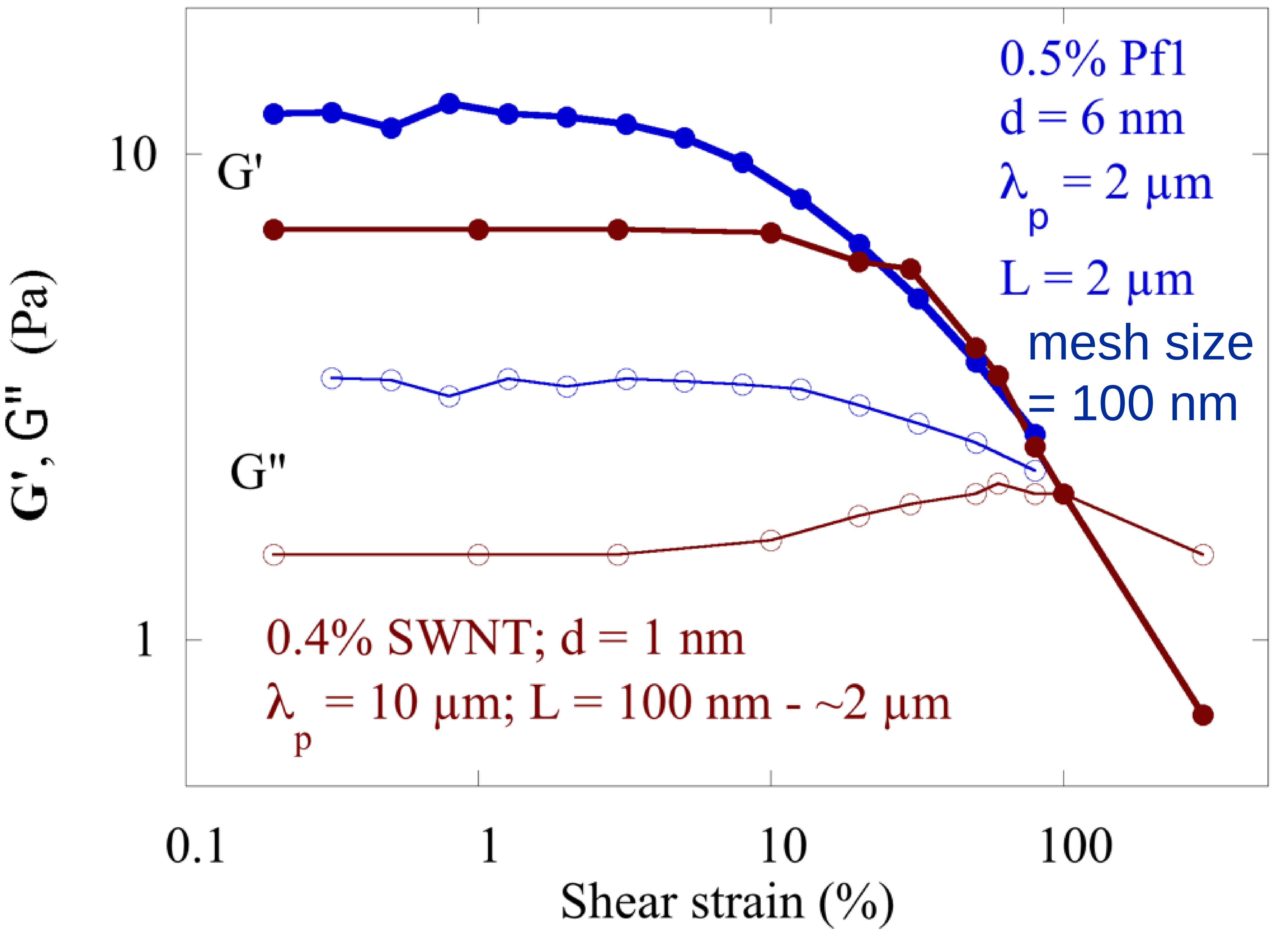
Viscoelasticity of Single Wall Carbon Nanotube Suspensions

L. A. Hough,[1] M. F. Islam,[1] P. A. Janmey,[2] and A. G. Yodh[1]



Rheology of Mn^{2+}-crosslinked Pf1 resembles that of VDW-crosslinked surfactant-stabilized carbon nanotubes



Conclusions

Nearly all cytoskeletal and nuclear polymers and filamentous viruses are strong polyelectrolytes

Release of intracellular polyelectrolytes is harmful because they bind and inactivate cationic antimicrobials by electrostatic interactions

Pf1 virus is good model system for network formation: monodisperse, semiflexible, anionic

Gels of polyelectrolytes can be formed by counterion concentrations too small to form bundles - crosslinks break at small strains, but rapidy reform